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The impact of long-term testosterone replacement therapy on lipid and lipoprotein profiles in women

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Abstract

The lipid and lipoprotein profiles of 39 female transsexuals, exposed to testosterone esters (250 mg monthly) for an average duration of 33 months after their sex reassignment operation (group 2), were compared to those of 29 normal menstruating female transsexuals prior to starting androgen therapy (group 1). A third group, comprising 17 post-operative female transsexuals were studied while on, and after stopping their androgen therapy for 6–12 months (group 3). The average concentration of testosterone in androgenized women was comparable to those found in normal males and levels of SHBG were significantly lower than those in the control group. No significant difference was noted between all levels of lipids and lipoproteins in pre-operative subjects of group 1 and corresponding levels in subjects of group 3 after they had stopped their androgen therapy for 6–12 months. Significantly higher levels of triglyceride (Trig), total cholesterol (TC), low-density lipoprotein (LDL-C) and apolipoprotein-B (Apo B) and a significantly lower level of high-density lipoprotein-cholesterol (HDL-C) were noted in androgenized women (group 2) when compared to controls (group 1). The two atherogenic indices, LDL-C/HDL-C and Apo-AI/Apo-B were significantly raised and lowered, respectively. Similar results were noted when comparing lipid and lipoprotein profiles in subjects of group 3 while they were on and after stopping their androgen therapy. Results from this study indicate that testosterone, per se, at supraphysiological doses may promote atherogenicity in women. Furthermore, the male predilection for coronary vascular diseases (CVD) may be due to the adverse effects of higher androgen levels on lipid and lipoprotein profiles.

Keywords: Androgen therapy; Lipids; Lipoproteins; Cholesterol; Coronary vascular diseases; Atherogenicity

1. Introduction

It is well established that the risk of mortality due to CVD in men is 5 times higher than healthy, normal menstruating women [1,2]. Such a sex difference has been attributed to beneficial effects of high levels of estrogens in women [3–6]. Whether high levels of testosterone is the reason for the male predilection for CVD remains unclear.

There are many mechanisms by which estrogens exert their beneficial effects on cardiovascular functions, among which are vasodilatory effects on

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muscles in the vessel walls. Estrogens can also decrease TC and LDL-C serum levels and increase HDL-C levels [7,8]. Since high levels of LDL-C concurrent with low levels of HDL-C are associated with increased risk of CVD [4], the cardioprotective effect of estrogens is believed to be due, in part, to their ability to induce quantitative and qualitative changes in lipid and lipoprotein profiles [3–6,9].

The risk of morbidity and mortality due to CVD in menopausal women is higher than normal menstruating women and approaches that of men [1,2,7,12,13]. Menopause in women results in a chronic state of estrogen-deficiency. Together with decreased levels of sex hormone binding globulin (SHBG), menopausal women are, therefore, exposed to a higher level of androgenic action than in their premenopausal state. Is the increased exposure to higher androgenic action in menopausal women contributing to the increased risk of CVD in women?

Although some studies had evaluated effects of synthetic androgens such as stanozolol and danazol [14], studies on the direct effect of long-term testosterone therapy on lipid and lipoprotein profiles in women have been difficult to carry out to date, due to the ethical problem of androgenization. It is mandatory for female transsexuals to undergo long-term androgen therapy, before and after their sex change surgery. Therefore, using the female transsexual model we were able to overcome this particular ethical hurdle and evaluate the long-term effects of supraphysiological doses of testosterone on lipid and lipoprotein profiles in women.

2. Materials and methods

2.1. Subjects

Three groups of female transsexuals were involved in the study. Group 1, a control group, consisted of 29 normal menstruating transsexuals who have had no previous history of hormone therapy. The mean (± S.E.) age of the female controls was 26.5 ± 0.78 years. Group 2 had 39 female transsexuals (age 31.7 ± 0.88 years) who have successfully gone through their sex reassignment operations, comprising total hysterectomy, bilateral oophorectomy, and the construction of a neophallus. They had been on long-term testosterone therapy for an average of 33 months before they were recruited into the study. Each patient was given monthly intra-muscular injections of 250 mg of a depot preparation of testosterone esters (Sustanon-250, Organon). Group 3 comprised 17 female transsexuals who had undergone their sex change operation 6 months to 6 years previously.

Serum lipid and lipoprotein profiles were determined on 2 occasions; once when subjects were on testosterone therapy for durations ranging from 6 months to 10 years and the other, after they had stopped their therapy for 6 to 12 months. The average age while they were on and after they had stopped androgen therapy was 26.9 (± 0.73) and 28.6 (± 0.68), respectively. Each subject in group 3 was also represented in groups 1 and 2. All subjects in each group were normo-tensive and had no other known illnesses. None of the subjects involved in this study were grossly obese or had any problem associated with body weight. The mean body mass index (BMI) was 22.9 (± 0.6) and 23.5 (± 0.5) for groups 1 and 2, respectively. Only two subjects in the test group were light cigarette smokers. From verbal enquiries, it was noted that there were no apparent changes in dietary behavior and pattern of exercise in subjects before and after their sex change operation.

2.2. Blood sampling and analyses of lipids and lipoproteins

Blood samples were collected from each subject of the 3 groups after an overnight fast of at least 12 h. Serum was separated from blood after allowing it to clot for at least 1 h at room temperature. HDL-C was extracted from fresh serum by a precipitation method [15,16]. Extracted fractions and sera were then stored at −80°C before analyses. Total cholesterol [17], Trig [18] and HDL-C [15] were determined using kits from Boehringer Mannheim. Serum levels of LDL-C were calculated using the Friedwald formula [16]. Serum apolipoproteins (Apo AI, Apo AII and Apo B) were measured by immuno-turbidimetric assay methods using kits from Boehringer Mannheim [19]. The intra-assay and inter-assay coeffi-

cients of variation of assays for triglyceride, total cholesterol, and HDL-C, within the ranges relevant to the study, were less than 3%, while those for apolipoprotein AI, AII and B were less than 5%.

Testosterone levels were measured by radioimmunoassay using reagents and methods from the World Health Organization Matched Reagent Program [20]. The intra-assay and inter-assay coefficients of variation were less than 10% and 15%, respectively. Serum sex hormone binding globulin (SHBG) levels were measured in 16 and 23 subjects of group 1 and 2, respectively. The kits for SHBG, using an immunofluorometric method, were purchased from Delfia (LKB, Finland). The intra-assay and inter-assay coefficients of variation were less than 10%. Student's *t*-test was used for statistical analyses of data.

3. Results

Mean (± S.E.) levels of testosterone in female transsexual controls (group 1), before treatment with androgen, was low (2.12 ± 0.1 nmol/l) and similar to those found in untreated women [21]. The testosterone therapy regimen used in our clinic (250 mg of testosterone esters per month) had resulted in exposure of female transsexuals to normal male levels of testosterone [22]. The mean testosterone level in group 2 subjects was 20.1 ± 2.0 nmol/l. The wide scatter of testosterone levels in treated female transsexuals (group 2) can be accounted for, in part, by uneven releases of testosterone from the depot preparation over the 4 week-intervals and that blood samples were collected randomly from patients without reference to a fixed time after they had their last injection. Serum levels of SHBG in group 2 (21.5 ± 1.9 nmol/l), compared to corresponding levels in group 1 (63 ± 3.3 nmol/l), were significantly reduced (*t*-test, $P < 10^{-6}$) as a result of castration and long-term androgen therapy.

Women who had been castrated and exposed to male levels of testosterone for long periods, showed significant changes ($P < 0.05$) in lipid and lipoprotein profiles. Androgenized women in group 2 had significantly higher ($P < 0.05$) levels of Trig, TC, LDL-C and Apo-B and significantly lower

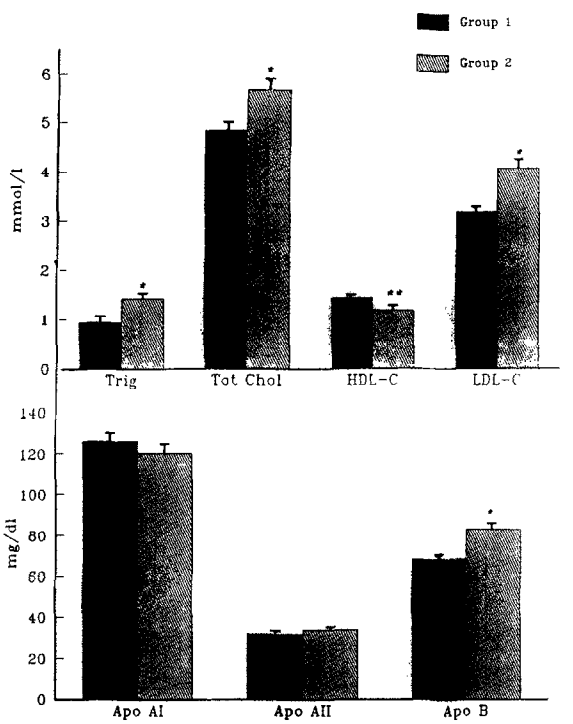

Fig. 1. Mean (± SE) concentrations of Trig, total cholesterol (Tot Chol), HDL-C, LDL-C, Apo-AI, Apo-AII and Apo-B in 29 female control transsexuals (group 1) and 39 castrated and androgenized female transsexuals (group 2). *Levels in subjects of group 2 were significantly higher ($P < 0.01$, *t*-test) than corresponding levels of subjects in group 1. **Levels of HDL-C of subjects in group 2 were significantly lower ($P < 0.01$, *t*-test) than corresponding levels in subjects of group 1.

levels of HDL-C when compared to pre-treated women of group 1 (Fig. 1).

Significantly higher ($P < 0.05$) levels of TC, LDL-C and Apo-B and significantly lower ($P < 0.05$) levels of Apo-A1 were noted when comparing levels in group 3 subjects while on, and after they had stopped, androgen treatment (Fig. 2). Slight differences in the intra-group 3 comparisons and those between groups 1 and 2 arose, probably as a result of the small number of cases in group 3. Levels of Trig, TC, HDL-C, LDL-C, Apo AI, Apo AII and Apo B in group 3 subjects after they had stopped androgen therapy for 6–12 months were not significantly different ($P > 0.05$) from corresponding levels in controls (group 1).

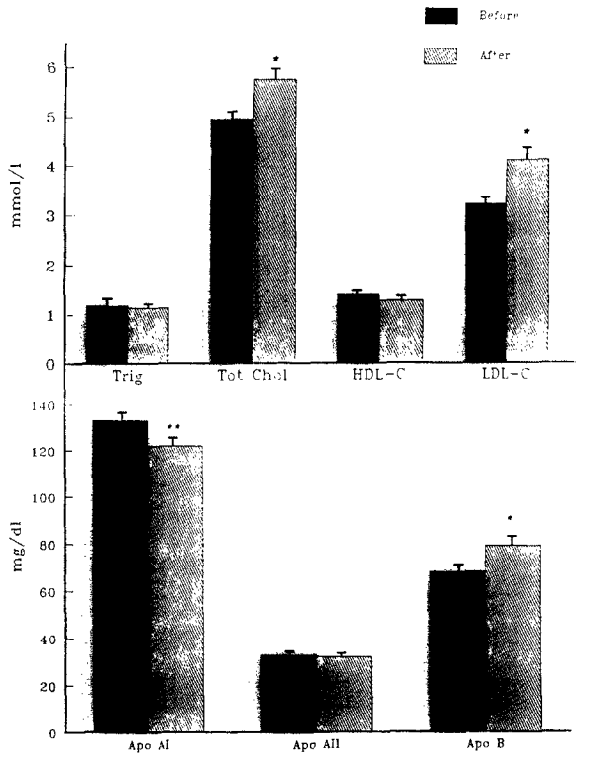

Fig. 2. Mean (± SE) concentrations of Trig, total cholesterol (Tot Chol), HDL-C, LDL-C, Apo-AI, Apo-AII and Apo-B in subjects of group 3 while they were on long-term hormone replacement therapy (HRT) and after they had stopped androgen therapy for 6–12 months. *Levels were significantly higher ($P < 0.01$, paired t-test) in subjects after HRT, compared to corresponding levels before HRT. **Levels in subjects after HRT were significantly lower ($P < 0.01$, paired t-test) than corresponding levels after stopping HRT.

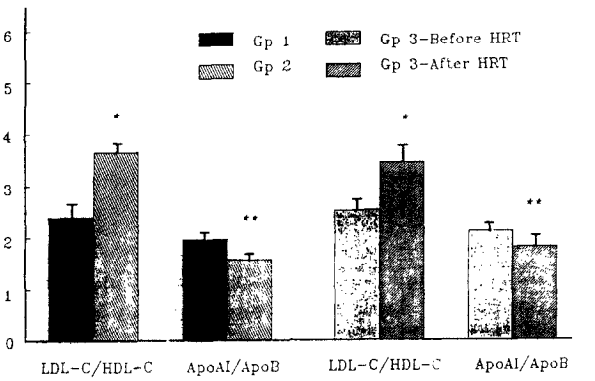

Fig. 3. Mean (± SE) of the atherogenic indices (LDL-C/HDL-C and Apo AI/ Apo B) in the three groups. *Values in subjects of group 2 and subjects in group 3 while they were on HRT were significantly higher ($P < 0.01$, paired t and t-tests) than corresponding values in group 1 and group 3 subjects after stopping HRT, respectively. **Values in subjects of group 2 and group 3 after HRT were significantly lower ($P < 0.01$, paired t and t-tests) than corresponding values in group 1 and group 3 subjects after stopping HRT, respectively.

Resulting from changes in lipid and lipoprotein profiles, the atherogenic indices, LDL/HDL and Apo AI/B, were significantly raised and lowered, respectively, in androgenized women (Fig. 3).

4. Discussion

Results from the present study showed that testosterone, per se, if administered at sufficiently high doses and for sufficiently long durations, can significantly raise TC, Trig, LDL-C and Apo-B and, at the same time, significantly lower HDL-C in androgenized women. In the cross-sectional comparison between group 1 and 2 subjects and the longitudinal comparison in group 3 subjects while on and after they had stopped androgen therapy, both atherogenic indices (LDL-C/HDL-C and Apo AI/Apo B) were significantly raised and lowered, respectively.

Oophorectomy and long-term androgen therapy are two possible reasons for the observed changes in lipid and lipoprotein profiles in post-operative androgenized transsexuals. If oophorectomy and not androgen were the major cause of the observed changes, then one would not expect any significant difference when comparing oophorectomized subjects in group 3 while they were on and after they had stopped their androgen therapy. On the contrary, significant changes in lipid and lipoprotein profiles were noted. Furthermore, the observation that lipid and lipoprotein profiles in non-oophorectomized and non-androgen treated females of group 1 were not significantly different from those of oophorectomized females who had stopped androgen therapy for 6–12 months, would suggest that oophorectomy, per se, is not the major cause of observed changes in androgenized women. This proposal was further supported when we showed that androgen therapy in non-oophorectomized subjects had induced a

similar trend of changes in lipid and lipoprotein profiles as observed in androgenized post-operative subjects of the present study. However, the small number of cases (3 subjects studied thus far), has limited the validity of the results (unpublished data).

We, therefore, proposed that changes in lipid and lipoprotein profiles in androgenized post-operative transsexuals are, probably, caused by long-term androgen therapy. Furthermore, chronic exposure to increased levels of testosterone may promote atherogenicity, as was also shown earlier for synthetic androgens [14]. Whether these changes in lipid and lipoprotein profiles would lead to higher atherogenic risk remains to be determined by a long-term follow-up of these subjects. Furthermore, how these testosterone-induced changes in lipid and lipoprotein profiles affect arterial wall thickening is currently being examined in a longitudinal study using intra-vascular ultrasound.

Our results were in contrast to the study using parenteral testosterone [14]. The observed differences could be due, in part, to the longer duration and higher levels of testosterone achieved in the present study [14].

The results also indicated that the higher risk for CVD in men as compared to women, i.e. the male predilection for CVD, may be due to adverse effects of higher androgen levels on lipid and lipoprotein profiles.

Menopause is associated with an increase in LDL-C and a decrease in HDL-C, leading to a subsequent increase in coronary heart disease which rapidly approaches the incidence observed in men of a similar age [1,2,12,13]. There are two possible reasons for this increased risk and incidence of coronary heart disease in menopausal women. Firstly, there is ample evidence showing that, with the drastic reduction of estrogens during menopausal state, the resistance to coronary disease is simultaneously reduced [1–3]. Secondly, we suggest that, concurrent with a reduction in estrogens and its attendant beneficial effects, the increase in androgenic action [21] may be contributory to the observed increase in LDL-C and decrease in HDL-C in postmenopausal women [1,2,12,13]. However, because the present study had evaluated the effect of supraphysiological doses of testosterone in women after surgical menopause, any extrapolation of findings to postmenopausal women must be viewed with caution.

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